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Integration Effect of Organic Manures and Phosphate Solubilizing Bacteria (PSB) on Phosphorus Availability and Release Kinetics in Sandy Calcareous Soil

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Abstract

A laboratory incubation experiment was conducted for periods (0, 3, 7, 15, 30, 45 and 60 days) to investigate the effect of organic wastes (poultry manure (PM), farmyard manure (FYM), and sheep manure (SM)) with and without phosphorus solubilizing bacteria (PSB) on soil pH, EC, available P, and CO₂ evolution as well as P release kinetics in a sandy calcareous soil. The treatments were arranged in a complete randomized design with three replicates. Soil moisture was maintained at field capacity throughout the incubation period. The organic manures (OM) were applied to soil at a level of 20 g kg⁻¹soil. The efficiency of *Bacillus megaterium* as a phosphate-solubilizing bacterium (PSB) was evaluated for its ability to convert insoluble P into soluble form in liquid culture. PSB demonstrated decreased pH and increased soluble P in the laboratory tests. The incubated soils' pH decreased with OM addition and increasing the incubation periods especially with PSB inoculation, the soil salinity was higher in the manure-amended soils. The CO_2 emissed as follows: PM > SM > FYM >control. Organic manures addition significantly increased soil available P at all incubation intervals especially with PSB inoculation. The PM + PSB-treated soil showed the highest available P content. The Parabolic diffusion model was the best for estimating P release in the amended soils, with higher release rate in the PSB-inoculated treatments. PM and SM yielded the highest counts of PSB. These findings highlight the dual role of organic amendments with PSB in improving phosphorus availability in sandy calcareous soil which reduces the need for the manufactured chemical phosphate fertilizers and support the sustainable agricultural production.

Key words: Sandy calcareous soil, Organic manures, Phosphorus solubilizing bacteria, Phosphorus availability, P release kinetics.

INTRODUCTION

Among the essential macronutrients for plant growth is phosphorus (P), it is the second important growth-limiting nutrient after nitrogen (N) (Adnan et al., 2019). According to Halajnia et al. (2009), mineral P in soil exists as phosphate anions that are either adsorbed to clay surfaces or form insoluble complexes with cations as Fe²⁺ and Al^{3+} in acidic soils or Ca^{2+} and Mg^{2+} in alkaline soils (Yadav et al. 2017). Phosphorus is highly immobile in soil, with only 15-25% of applied fertilizer P typically available to plants in the year of application (Deng et al., 2018). Thus, the bio-available P varies widely depending on soil type, management, and environmental conditions, but it is always a very small fraction of the total soil P (Xu et al., 2022). Approximately 80% of phosphate fertilizers used annually worldwide are lost as a result of precipitation, adsorption, and immobilization processes in soil (Gyaneshwar et al. 2002; FAO 2017). This pollutes the environment in addition to raising manufacturing costs (Tilman et al. 2001). Calcareous soils, which are rich in calcium carbonate (CaCO₃) and typically have a high pH, are well known for their tendency to "fix" phosphorus (P), making it less available to plants (Bolan et al., 2023). Phosphorus is mostly fixed in calcareous soils by adsorption and precipitation interactions with magnesium (Mg²⁺) and calcium (Ca²⁺) ions. These processes produce calcium phosphate minerals that are insoluble and difficult for plants to absorb (Adnan et al., 2025). Moreover, calcareous soils' high pH conditions encourage phosphorus to precipitate into these insoluble forms, decreasing its solubility and, consequently, plant availability (Alghamdi et al., 2023). The high concentration of free CaCO₃ in these soils increases the immobilization of phosphorus as low-solubility calcium compounds (Amin and Mihoub 2021; Jalali & Jalali, 2022). While inorganic P fertilizers (like DAP and SSP) provide immediate P availability, their efficiency in calcareous soils is often limited by rapid fixation. Particularly in alkaline or calcareous soils, the integration of organic manures and phosphate-solubilizing bacteria (PSB) improves plant development phosphorus and (P) availability (Adnan et al., 2022). Organic

manures alone provide a slower, more sustained release of P, but their total P content is generally lower than inorganic fertilizers (Padghan et al., 2024). When organic manures break down and produce organic acids, they can chelate calcium ions and lower the pH of the soil, which decreases the precipitation of P as insoluble Ca-P compounds and increases P mobility and solubility (Jamal et al., 2023). Poultry manure, sheep manure, and farmyard manure are abundant natural sources of nutrients (N, P, K) for agriculture. phosphate solubilizing bacteria (PSB) contribute significantly to the soil P cycle and increase P availability by participating in dissolution-precipitation, sorption-desorption, and mineralization-immobilization reactions (Jiang et al. 2018). They produce various organic acids, causes the soil to become more acidic (Penn and Camberato 2019), which in turn causes P to be released from Ca_3 (PO₄)₂ in calcareous soils. Via ligand exchange processes, these acids also displace adsorbed phosphate. can Additionally, organic acids may enhance the amount of P accessible to plants by chelating cations such Ca^{2+} , Al^{3+} , and Fe^{3+} (Jones 1998). When labile carbon is present, these bacteria may serve as a sink for P by quickly immobilizing it (Bünemann et al. 2004). When they break down, the P is released into the soil. Other ways that PSB enhance crop development and soil P nutrition include alkaline phosphatases (Rodriguez et al. 2002), H⁺ protonation (Xiao et al. 2017), anion exchange, chelation, siderophores, hydroxyl ions, and CO₂ production (Sugihara et al. 2010; Iqbal et al. 2019). Our hypothesis is that combined application of PSB and organic manures could be an efficient approach for improving P availability. Thus, this study aimed to 1- assess the potential of PSB in enhancement of P availability from different organic manures in sandy calcareous soil and 2- study the PSB effect on P release kinetics from the used organic manures in laboratory incubation experiment for 60 days.

MATERIALS AND METHODS

1. The used soil in the experiment

From the Experimental Farm (El-Kawthar farm) of the Faculty of Agriculture, Sohag University, Sohag, Egypt, a surface sample

of sandy calcareous soil (0-30 cm) was collected. To prepare it for the experiment and analysis, the obtained soil was thoroughly mixed, allowed to air dry, and then sieved using a 2 mm sieve. The physical and chemical characteristics were analyzed according to Jackson (1973). The soil particle size analysis showed that the texture of the used soil is a sandy predominantly consisting of 89.11 % sand, 5.74% silt and 5.19 % clay with pH (1:1) 7.98, EC_e (dS m⁻¹) 3.03, total CaCO₃ 11.94 %, OM 0.78 %, available P 5.42 mg kg⁻¹, and total P 393.26 mg kg⁻¹.

2. The applied organic manures

Three organic manures were used in this study included Poultry manure (PM), sheep manure (SM), and farmyard manure (FYM). These manures were obtained from the animal production and poultry farms, Faculty of Agriculture, Sohag University.

3. Organic manures analysis

The organic manure's pH was measured in 1:10 ratio suspension (manure: distilled water w/v) using pH meter with glass electrode (pH 211, Microprocessor, HANNA Instruments), then the suspension was filtered to measure the EC in the extract using the electrical conductivity meter (Jenway 4520 Conductivity, UK) (Schlichting et al., 1995). The total OM content in the organic manures was determined using loss on ignition method using muffle oven at 550 C. A 0.2 g of each ground dried organic manure was digested in 10 ml of 7:3 ratio sulfuric to perchloric acids mixture and then analyzed for the total content N (%), P (%) and K (%) according to Jackson (1973). Nitrogen was determined using Kieldahl (Automatic distillation system Rapidstill II, Labconco, USA) equipment (Jackson, 1973). The molybdate-ascorbic acid colourimetric method was used for P determination by a visible spectrophotometer (BK-UV 1900, China) at 880 nm (Murphy and Riley, 1977). Potassium was measured by the flame photometer (CL 378 -ELICO) (Jackson, 1973). Table 1 presents some chemical properties of the used organic manures.

Property	Unit	Poultry manure (PM)	Farmyard manure (FYM)	Sheep manure (SM)		
pH (1:10)	I	6.43	7.86	8.04		
EC (1:10)	dS m ⁻¹	5.16	5.49	6.68		
OM		85.59	50.09	65.63		
OC		49.64	29.05	38.07		
Total N	%	5.04	1.21	1.39		
Total P		2.07	1.75	1.70		
Total K		2.54	1.33	2.83		

Table 1. Chemical properties of the used organic manures.

4. The bacterium strain:

An identified isolate of phosphorussolubilizing bacteria (*Bacillus megaterium*) was supplied from the stock culture of the laboratory of microbiology at the Faculty of Agriculture, Sohag University, Sohag, Egypt.

5. Determination of the efficiency of used bacteria:

The bacterium was grown in Bunt and Rovira (1955) liquid medium. Ninety ml aliquots of the medium were placed in 250 ml Erlenmeyer flasks. Tri-calcium phosphate was added to flasks at the rate of 0.25 g, and pH was adjusted at 6.8, and then the flasks were autoclaved. Flasks were inoculated with the tested microorganism and incubated at 33 ± 2 °C. Changes in pH were measured at intervals of 0 days up to 7 days. The quantity of soluble phosphorus found was taken as an indicator for the efficiency of the bacterial isolate.

6. Bacterial inoculum:

Bacillus megaterium isolate was grown in 250 ml Erlenmeyer flasks containing nutrient broth medium (200 ml/flask) for four days at $33\pm2^{\circ}$ C. These liquid cultures were used as a bacterial phosphate dissolver inoculum (Zayed 1997). The growth rate of the bacteria strain was calculated and reported as log_{10} CFU/mL. At least 5×10^{11} CFU mL⁻¹ were present in the strain.

7. The incubation experiment set up:

60-day laboratory Α incubation experiment using a complete randomized block design was conducted to assess the changes of soil pH, EC, and soil available phosphorus (P), and the release of CO₂ in sandy calcareous soil treated with various organic manures, either with or without phosphorus-solubilizing bacteria inoculation. Each of the studied organic manures was thoroughly mixed with 100 g of the soil sample at a level of 2% in glass jars. There were six replications of each treatment, three of which were inoculated with the phosphorus-solubilizing bacteria (1 ml) and the other three of which were not. The experiment also included a control treatment without organic manures addition. Throughout the experiment, the soil moisture content in each jar was maintained at its field capacity. For 60 days, all treatments were incubated at room temperature (23 ±2°C) (A thermometer was hung in a burette holder to record the laboratory temperature daily, and the average temperature was taken during the incubation period).

8. Soil analysis:

The soil samples of each treatment at the end of each incubation periods of 0, 3, 7, 15, 30, 45 and 60 days were taken to determine soil pH in 1:1 suspension, electrical conductivity (EC) in 1:1 extract, the evolved CO₂ and the released P (available P). The available P content was extracted with sodium bicarbonate solution (0.5 M, pH 8.5) based on Olsen et al. (1954) method. The soil available P content was determined using the molybdate-ascorbic acid colourimetric method by a visible spectrophotometer (BK-UV 1900, China) at 880 nm (Murphy and Riley, 1977).

9. Soil respiration (Carbon dioxide (CO₂) trapping):

For measuring the released CO_2 from treated soils at every incubation period, vials contain 10 ml of 1 M NaOH solution were put inside vessels filled with 100 g soil of each treatment having field capacity moisture level. After that, the vessels were closed tightly and incubated under the same room temperature conditions. The NaOH solution in the vials was taken after an incubation period of 0, 3, 7, 14, 30, 45 and 60 days. For 0 day period, the NaOH solution in the vials was taken after 2 hr. The CO₂ evolved during each incubation period was trapped in NaOH (1M) and the excess of NaOH was titrated with HCl (0.1 M) after adding barium $(BaCl_2)$ (0.5 M) solution chloride and phenolphethaline indicator. Mineralized C along incubation experiment was calculated as a cumulative CO₂- evolution (mg g^{-1} soil) according to Rubio (2017).

10. Kinetics of phosphorus (P) release

Each soil's cumulative P release from the treated soils was plotted against time and fitted using the linear forms of six kinetic models, included zero order, first-order, second-order, power-function, simplified Elovich and parabolic diffusion equations (Laidler 1965, Chien and Clayton 1980, Dang et al., 1994; Suwanree et al., 2022; Kataria & Singh, 2024), as follows:

Zero order equation: $C_A = C_{A0} + K_0 t$ (1) First order equation: $\ln C_A = \ln C_{A0} + K_1 t$ (2) Second order equation: $1/C_A = 1/C_{A0} + K_2 t$ (3) Power function equation: $Ln C_A = \ln k_f + b \ln t$ (4) Simplified Elovich equation: $C_A = a + \beta \ln t$ (5) Parabolic diffusion equation: $C_A = kp t^{0.5} + a$ (6)

Where:

 C_A is the amounts of P released (mg kg⁻¹) after incubation time t (days); and C_{A0} is the amounts of P released (mg kg⁻¹) at t = 0, respectively; K_0 , K_1 , and K_2 are the zero-order, first-order, and second-order release rate coefficients; k_f is the release rate coefficient of power function model, b is constant; β is the release rate coefficient of simplified Elovich model, α is the initial release rate constant; kp is the release rate constant of Parabolic diffusion model; *a* is constant.

11. In the In vivo Study:

Numbers of P-dissolving bacteria were estimated at the end of the experiment in the soils as described by Abdel-Moniem *et al.* (1988). Ten grams of each collected sample were placed into a sterile homogenizer flask with 90 milliliters of 0.1% sterile peptone water. The contents were homogenized for two to four minutes at 1400 r.p.m. and then left to stand for five minutes at room temperature to create the first serial dilution, 10^{-1} . The contents of the flask were thoroughly mixed by shaking, and one milliliter was transferred into a separate sterile test tube with 9 milliliters of 0.1% sterile peptone water to create the second serial dilution, 10^{-2} , and so on to the dilution of 10^{-10} . Bacterial populations were estimated using the standard plate count method on selective media (Bunt and Rovira 1955) specific for phosphate-solubilizing bacteria after incubation for three days at $33\pm2^{\circ}$ C.

12. Statistical analysis:

At each incubation period, the Duncan multiple range tests at a 5% level of probability and a one-way ANOVA analysis of variance using SPSS IBM version 22 software were used to examine the significance differences between the treatments.

RESULTS AND DISCUSSION

1. Efficiency of used bacteria:

The liquid cultures of *Bacillus megaterium* isolate as phosphate solubilizing bacteria were tested for their ability to dissolve insoluble phosphate, and the results showed a decrease in pH values and a rise in soluble phosphorus.

Following seven days of incubation at $33\pm2^{\circ}$ C, the pH values in the liquid cultures of the *B*. *megaterium* isolate gradually dropped, as indicated by the data in Table (2). An increase in soluble phosphorus was associated with a drop in pH values. The liquid culture of the *B*. *megaterium* isolate had low pH values, which led to the observation of high soluble phosphate concentrations. Thus, this *B. megaterium* isolate was chosen to serve as an inoculum that dissolves phosphate in the soil experiment, such finding coincides with that obtained by Taha et al. (1969), Zayed (1997) and Liu et al. (2015).

Table (2): Soluble-phosphorus (P) concentrations (ppm) and pH values in the liquid cultures of *B*. *megaterium* isolate as phosphate dissolving bacteria.

	Days after incubation at 33°C														
	0		1		2	(1)	;	4	ł	4	5		6	7	1
Р	pН	Р	pН	Р	pН	Р	pН	Р	pН	Р	pН	Р	pН	Р	pН
21	7.2	80	6.20	97	5.6	109	5.2	117	4.8	136	4.3	142	3.93	148	4.2

2. Effects of organic manures and PSB additions on some soil chemical properties 2.1. Soil pH

For sustainable crop production, nutrient availability optimization, and the maintenance of healthy microbial populations, proper soil pH management is essential. The reduction in calcareous soil pH between neutral to slightly alkaline pH enhances the nutrients availability especially phosphorus and encourage the soil microbes involved in organic matter decomposition and nutrient mineralization. The obtained results indicated that the pH of the soil significantly decreased by applying the studied organic manures, either with or without the PSB inoculation in comparing with control (CK) at all incubation periods (Table 3). Moreover, the soil pH decreased with increasing incubation time. The type of organic manure and how it decomposed affected the reduction in soil pH.

The application of poultry manure (PM), which has the lowest pH among all utilized organic manures (6.43), produced the lowest soil pH throughout the incubation period, values beginning with 7.77 and 7.80 at 0 day and ending at 7.43 and 7.58 after a 60-day incubation period, with or without PSB addition, respectively. Moreover, the treated soil with FYM showed soil pH values starting with 7.88 and 7.91 at 0 day and ending with 7.56 and 7.74 after 60 days of incubation with or without PSB addition, respectively. However, the sheep manure (SM) treatment recorded higher soil pH values than PM treatment; starting with 7.97 and 7.99 at 0 day and ending with 7.62 and 7.76 after 60 days of incubation with or without PSB addition, respectively. At the end of incubation (60 days), the soil pH decreased by 0.29, 0.13, and 0.11 unit in PM-, FYM-, and SM-treated soil, respectively compared to CK (Table 3). The integrated use of PSB with organic manures lowered soil pH significantly at all the incubation periods except zero period. The PSB application decreased pH throughout the incubation period by 0.11-0.22, 0.10-0.20, 0.09-0.23 unit in PM+PSB, FYM+PSB, and SM+PSB treatments, respectively compared to PM, FYM, and SM treatments. At the end of incubation period (60 days), the soil pH decreased by 0.37, 0.24, and 0.18 in PM+PSB, FYM+PSB, and SM+PSB respectively compared to CK+PSB. This indicates that PSB be more efficient in decreasing soil pH in presence of the organic manures, which may be that organic manures provide a rich source of carbon and nutrients, which stimulate the growth and activity of PSB (Fouda, 2020). Generally, the lowest pH values for PM and PM+PSB treatments (7.58, 7.43) were recorded at 60 days of incubation, while the lowest pH values for FYM and FYM+PSB treatments (7.69, 7.50), as well as for SM and SM+PSB treatments (7.77, 7.54) were recorded at 30 days of incubation. This indicates the higher effectiveness of PM in reducing soil pH for a longer period either with or without inoculation with PSB (Table 3). Organic acids, including acetic, citric, and malic acids, are produced when microbes break down complex organic components in organic manures. As a result of these acids' dissociation, hydrogen ions (H⁺) are released into the soil, lowering its pH (Nivethadevi et al., 2021). CO₂ is produced during the decomposition of the organic matter by intense microbial activity. Carbonic acid, which is created when CO₂ dissolves in soil water, can further reduce the soil pH (Ferdush & Paul, 2021). Soil pH gradually decreased with the increase of incubation time as decomposition progresses (Roy & Kashem, 2014; Ragheb et al., 2018). Our results agreed with Walpola & Hettiarachchi (2018) and Thite et al. (2022) findings. PSB further enhances this acidification process, as they produce low molecular weight organic acids (such as gluconic, oxalic, and citric acids) (Iftikhar et al., 2023). Organic manures provide a rich substrate that supports the proliferation and activity of PSB (Zhang et al., 2023), so the combined application of PSB and organic manures showed the best effect in reducing soil pH in all treated soils with some variations related to the manure type. Similar results were obtained by (Adnan et al., 2022).

2.2. Soil salinity

Soil EC is a measure of soluble salts (nutrients) concentration in the soil. As organic manures decompose, they release these nutrients into the soil solution, thereby raising EC. In general, results showed that the examined organic manures could be arranged depending on their effect on soil EC (1:1) increase as follows: sheep manure > farmyard manure > poultry manure. After 60 days of incubation, the addition of sheep manure, farmyard manure, and poultry manure raised soil salinity (EC) significantly to 4.03, 3.72, and 3.46 dS m⁻¹, respectively, compared to the control (2.72 dS m^{-1}) (Table 3). The soil EC increase is linked to the soluble nutrients release during manure decomposition and the salt content of manure itself. These results are in agreement with Awad et al. (2015), Ragheb et al. (2018) and Tlass et al. (2024). There was a slight and nonsignificant increase in soil EC in the PSBinoculated soils compared to the uninoculated ones, this may be due to that PSB release organic acids and other metabolites, which may contribute to increased EC by raising soluble ion concentrations in the soil (Namli et al., 2017).

	Incubation periods (days)								
Treatments	0	3	7	15	30	45	60		
			So	il pH (1:1)				
CK	7.96 a	7.94 a	7.88 a	7.86 a	7.83 a	7.85 a	7.87 a		
CK+PSB	7.95 a	7.86 bc	7.83 b	7.80 b	7.77 b	7.80 b	7.81 b		
PM	7.80 c	7.78 d	7.77 c	7.72 c	7.68 c	7.62 d	7.58 e		
PM+PSB	7.77 c	7.67 e	7.56 f	7.50 f	7.47 e	7.46 g	7.43 f		
FYM	7.91 b	7.89 b	7.78 c	7.74 c	7.69 c	7.73 c	7.74 c		
FYM+PSB	7.88 b	7.79 d	7.65 e	7.58 e	7.50 e	7.53 f	7.56 e		
SM	7.99 a	7.93 a	7.87 a	7.83 ab	7.77 b	7.75 c	7.76 c		
SM + PSB	7.97 a	7.84 c	7.71 d	7.64 d	7.54 d	7.58 e	7.62 d		
			Soil EC	C (1:1) (ds	5 m ⁻¹)				
CK	2.28 d	2.32 d	2.40 d	2.53 d	2.63 f	2.67 f	2.72 d		
CK+PSB	2.33 d	2.40 d	2.53 d	2.69 d	2.82 e	2.90 e	2.89 d		
PM	2.79 c	2.93 c	3.02 c	3.15 c	3.29 d	3.37 d	3.46 c		
PM+PSB	2.86 bc	3.04 bc	3.18 bc	3.31 bc	3.47 c	3.60 c	3.71 b		
FYM	2.87 bc	2.97 c	3.14 bc	3.29 bc	3.48 c	3.59 c	3.72 b		
FYM+PSB	2.94 abc	3.08 bc	3.26 b	3.44 b	3.65 bc	3.71 bc	3.85 b		
SM	3.00 ab	3.21 ab	3.48 a	3.66 a	3.80 ab	3.88 ab	4.03 a		
SM+PSB	3.09 a	3.29 a	3.61 a	3.74 a	3.90 a	4.01 a	4.09 a		

Table 3. Effect of the applied organic manures and PSB on soil pH and salinity (EC) (the different lowercase letters beside mean values indicate significant differences based on Duncan multiple range tests at $p \le 0.05$).

2.3. Soil available P

Organic manures serve as a crucial source of plant nutrients, providing both macroand micronutrients while improving soil health. Derived from plant, animal, and industrial byproducts, they release nutrients gradually via microbial decomposition. In this study, organic manures addition, both with and without the PSB, caused gradual and significant increase in the soil available P driven by microbial mineralization and acidification (Walpola and Hettiarachchi, 2020). The lowest available p values were observed in control soil that did not receive organic manure (Table 4). Amending soil with the organic manures significantly increased soil available P content compared to control at all incubation periods. Also, the available P content increased with the increase of incubation time. The available P values ranged from 5.30 to 11.35 mg kg⁻¹ in control soil, while in the PM-amended soil it ranged from 10.29 to 43.59 mg kg⁻¹.

Moreover, the FYM-amended soil recorded available P values ranged from 20.30 to 36.06 mg kg⁻¹, while the available P ranged from 17.60 to 37.16 mg kg⁻¹ in SM-treated soil. The P release from organic manures was rapid till 30 or 45 days of incubation then the release rate decreased or stabilized (Table 4). For CK soil, the maximum available P (11.35 ppm) was at 30 days of incubation. In PM-treated soil, it reached its maximum level (43.59 mg kg⁻¹) at 60 days. For and FYM-treated soil, the maximum P content $(36.06 \text{ mg kg}^{-1})$ was at 30 days of incubation. while in the SM-treated soil, the maximum available P (37.16 mg kg⁻¹) was at 45 days of incubation. At 60 days of incubation (the incubation end), the soil available P content increased from 9.76 mg kg⁻¹ in CK to 43.59, 34.05, and 35.03 mg kg⁻¹ in the PM-, FYM-, and SM-amended soils, respectively.

Incubation periods (days)							
0	3	7	15	30	45	60	
5.30 e	6.82 f	8.73 g	9.92 e	11.35 e	10.91 e	9.76 f	
5.71 e	8.75 e	11.36 f	12.16 d	14.08 d	13.02 e	11.57 e	
10.29 d	13.07 d	17.19 e	28.33 c	36.54 b	40.49 b	43.59 b	
11.41 d	18.87 c	26.65 c	36.04 a	42.38 a	47.92 a	49.74 a	
20.30 ab	23.36 b	25.92 с	28.45 c	36.06 b	33.86 d	34.05 d	
21.17 a	27.39 a	30.26 a	34.46 a	41.32 a	39.87 b	40.49 c	
17.60 c	19.86 c	22.92 d	27.45 c	31.19 c	37.16 c	35.03 d	
18.66 bc	23.28 b	28.26 b	30.58 b	35.66 b	40.89 b	39.54 c	
	5.30 e 5.71 e 10.29 d 11.41 d 20.30 ab 21.17 a 17.60 c	5.30 e 6.82 f 5.71 e 8.75 e 10.29 d 13.07 d 11.41 d 18.87 c 20.30 ab 23.36 b 21.17 a 27.39 a 17.60 c 19.86 c	0375.30 e6.82 f8.73 g5.71 e8.75 e11.36 f10.29 d13.07 d17.19 e11.41 d18.87 c26.65 c20.30 ab23.36 b25.92 c21.17 a27.39 a30.26 a17.60 c19.86 c22.92 d	037155.30 e6.82 f8.73 g9.92 e5.71 e8.75 e11.36 f12.16 d10.29 d13.07 d17.19 e28.33 c11.41 d18.87 c26.65 c36.04 a20.30 ab23.36 b25.92 c28.45 c21.17 a27.39 a30.26 a34.46 a17.60 c19.86 c22.92 d27.45 c	03715305.30 e6.82 f8.73 g9.92 e11.35 e5.71 e8.75 e11.36 f12.16 d14.08 d10.29 d13.07 d17.19 e28.33 c36.54 b11.41 d18.87 c26.65 c36.04 a42.38 a20.30 ab23.36 b25.92 c28.45 c36.06 b21.17 a27.39 a30.26 a34.46 a41.32 a17.60 c19.86 c22.92 d27.45 c31.19 c	0371530455.30 e6.82 f8.73 g9.92 e11.35 e10.91 e5.71 e8.75 e11.36 f12.16 d14.08 d13.02 e10.29 d13.07 d17.19 e28.33 c36.54 b40.49 b11.41 d18.87 c26.65 c36.04 a42.38 a47.92 a20.30 ab23.36 b25.92 c28.45 c36.06 b33.86 d21.17 a27.39 a30.26 a34.46 a41.32 a39.87 b17.60 c19.86 c22.92 d27.45 c31.19 c37.16 c	

Table 4. Effect of the applied organic manures and PSB on soil available P content (mg P kg⁻¹ soil) (the different lowercase letters beside mean values indicate significant differences based on Duncan multiple range tests at $p \le 0.05$).

The net increase in the soil available P over the incubation (the difference between available P content at 0 and 60 days) was 4.46, 33.30, 13.75, and 17.43 mg kg-1 in CK, PM, FYM, and SM treatments, respectively. Thus, the high P content and its bioavailability make poultry manure an effective P fertilizer in sandy calcareous soils more than the other manures. Manures contain both organic and inorganic forms of phosphorus, with a significant portion being labile for plant uptake. Almost of the total P in manure is considered labile, making manure a direct source of plant-available P (Sun et al., 2022). Manure's organic materials have the ability to lessen P's adsorption, or binding, to soil particles. By competing with phosphate ions for adsorption sites on soil colloids, organic acids released during manure decomposition increase the solubility and mobility of P in the soil (Khan et al., 2022). Applying manure increases soil microbial activity by supplying readily decomposable carbon sources. The transformation of organic P into inorganic forms that plants can absorb is accelerated by this enrichment of microbial populations, particularly those that can mineralize organic P (Sun et al., 2022). The decomposition of organic materials and the production of organic acids may be the cause of the increase in soil phosphorus availability brought on by their application. It might also be due to the P release after decomposition of these organic materials. Moreover, the phosphate dissolving bacteria that increases the soil available P. Our findings agreed with Jalali et al. (2023) who indicated that incubation soil with 200 mg P kg in from poultry

manure for 3 weeks increased the Olsenextractable P from 32.68 mg P kg⁻¹ in control soil to 86.85 mg P kg⁻¹ in PM-treated soil. After 4 weeks of incubation, Kashem et al (2004) indicated that the Olsen-extractable P increased from 13 mg P kg⁻¹ in control soil to 28, 64, 117, and 262 mg p Kg⁻¹ in the amended soil with 110, 220, 440, and 880 mg P kg⁻¹ soil in the form of cattle manure (contains 0.55 % total P). Moreover, Ara et al. (2018) proved that amending an alkaline soil with 200 mg P kg⁻¹ soil in the form of cow manure (CM) and poultry manure (PM) (based on their content of total P) increased the Olsen-extractable P from 9 mg P kg⁻¹ soil in control to 67 and 125 mg P kg⁻¹ soil in the CMand PM-amended soils, respectively at 60 days of incubation. In similar study and on DAI 70 (days of incubation), Wang et al. (2023) found that the fractions of labile P (Resin-P + NaHCO₃-Po + NaHCO₃-Pi) in soils amended with poultry manure (PM), and cattle manure (CM) increased by 64.2 and 97.0 mg P kg⁻¹ soil, respectively compared with control. Another study by Ragheb et al. (2018) found that amending sandy soil with compost, filter mud cake, farmyard manure, and poultry manure at level of 3 % increased the available P content after 45 days of incubation from 14.82 mg P kg⁻¹ in control to 35.93, 51.01, 46.26, and 50.16 mg P kg⁻¹, respectively.

Regarding to the results in Table (4) and comparing with CK, PM, FYM and SM-treated soils, inoculation with PSB increased available phosphorus in CK+PSB, PM+PSB, FYM+PSB, and SM+PSB-treated soils by 28.30, 44.38, 17.25, 17.22 % at 3 days of incubation, and by 30.13, 55.03, 16.74, and 23.30 % at 7 days of

incubation; and by 22.58, 27.21, 21.12, and 11.40 % at 15 days of incubation (Table 4). Similar trend was observed at the next periods of incubation, where the available phosphorus increased in CK+PSB, PM+PSB, FYM+PSB, and SM+PSB treated soils by 24.05, 15.98, 14.59, and 14.33 % at 30 days of incubation, and by 19.34, 18.35, 17.75, and 10.04 % at 45 days of incubation, and by 18.55, 14.11, 18.91, and 12.87 % at 60 days of incubation, respectively compared to the un-inoculated soils (CK, PM, FYM, and SM treated soils) (Table 4). Poultry manure (PM), with its higher phosphorus content. showed the greatest phosphorus availability when combined with PSB (49.74 mg kg⁻¹). Similar results were obtained by Walpola and Hettiarachchi (2018), Adnan et al. (2019), Ahmed et al. (2025). The Bacillus megaterium (PSB) secretes organic acids and enzymes (e.g., phosphatases, phytases) that mineralize the organic P to inorganic P and dissolve insoluble phosphorus compounds like tricalcium phosphate and increases P availability. Organic acids from PSB and manure chelate cations (e.g., Ca²⁺, Al³⁺, Fe³⁺), preventing phosphorus fixation into insoluble forms. This process is particularly effective in alkaline-calcareous soils, where calcium binds phosphorus. This effect is amplified when combined with organic manures, which provide additional substrates for microbial activity. With the use of organic manures, PSB viability rose. This may also be explained by how decomposition releases nutrients like C and N. which encourage the growth of soil bacteria (Nardi et al. 2017). Our results are in agreement

with Walpola & Hettiarachchi (2018) who found that combining organic manures (like poultry manure (PM), cow dung (CD), or goat manure (GM)) with phosphate solubilizing bacteria (PSB) significantly increased soil P availability at 56-days incubation period from 50.21, 42.37, 47.66 mg P kg⁻¹ soil in PM-, CD-, and GM-treated soils to 62.24, 51.56, and 53.34 mg P kg⁻¹ soil in PM+PSB-, CD+PSB-, GM+PSB-treated ones, respectively.

2.4. Carbon dioxide evolution:

Adding organic manures to soil generally increased soil CO₂ emission significantly compared to control (CK). Figure (1) showed the total amount of the released CO2 from each treatment, and Figure (2) showed the cumulative amount of released CO₂ with incubation periods. The total amount of released CO₂ from the soils treated with organic manures differed according to the manure type, and increased from 3.57 mg CO_2 g⁻¹ soil in control to 10.19, 6.72, and 7.75 mg CO₂ g⁻¹ soil in the PM-, FYM-, and SM- treated soil, respectively. Phosphorus solubilizing bacteria inoculation increased the total CO2 emission non-significantly to 3.81, 10.38, 7.15, and 8.17 mg CO₂ g⁻¹ soil in CK, PM+PSB, FYM+PSB, and SM+PSB treatments. respectively. The rise in CO₂ emissions is primarily because of the microbial decomposition of the applied organic manures. Soil microbes decompose the carbon-rich molecules in manure, and CO₂ is released as a byproduct of respiration (Liu et al., 2024).

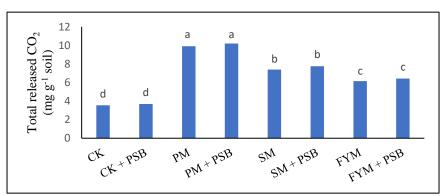


Fig. (1): Effect of organic manures and PSB on the total amount of released CO_2 during the incubation period (the different lowercase letters above columns indicate significant differences based on Duncan multiple range tests at p ≤ 0.05).

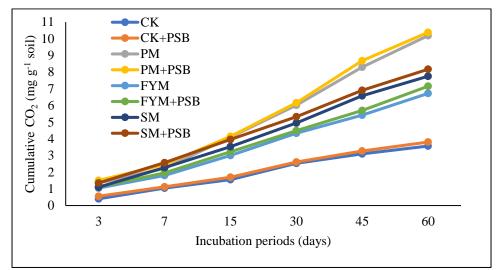


Fig. (2): The cumulative CO₂ release from treated soils at incubation periods.

2.5. Kinetics of phosphorus release

To give a quantitative description of the P release mechanism, a mathematical model must be created. To provide mutual validation between the empirical formula and the experimental results, the ideal formula or model must be found. The kinetic equations (zero-, first-, second-, Elovich-, power function-, and parabolic diffusion-model) characterized the P release against time in amended soils by organic manures and PSB. Table (5) displays linear regression equations (forms) of the kinetic models. Table (6) showed the rate constants that resulted from these equations and the determination coefficients (R^2) for the kinetic equations. Based on R^2 values, Phosphorus release in CK and CK+PSB treated soils was best described by Power function model $(R^2 = 0.8810, 0.9034)$. For the organic manures treated soils, the Parabolic diffusion model was the best one for fitting the P release either with or without PSB inoculation. The R² values of Parabolic equation were 0.9700 and 0.9744 in PM and PM+PSB treated soils, 0.8835 and 0.9106 in FYM and FYM+PSB treated soils, and 0.9564 and 0.9668 in SM and SM+PSB treated soils, respectively (Table 6). Similar results were obtained by Toor & Bahl (1999) and Kataria & Singh (2024). The parabolic kinetic model assumes an initial rapid release phase followed by a slower, diffusion-controlled process. Vanitha et al. (2020) reported that most of the increase in accessible soil P from organic manure happens in the first two to three weeks following application.

The available P gradually decreases or stabilizes after this initial flush when the readily mineralizable portions are depleted. The higher microbial activity within the first few weeks after manure application pronounce the P release and availability. The abundance of microorganisms in manure-amended soils, especially when combined with phosphate-solubilizing bacteria (PSB), accelerates the mineralization of organic P to inorganic forms, further increasing P availability within the first weeks (Vanitha et al., 2020). Because they assume a constant or proportional rate of release, zero-, first-, and second-order kinetic models did not adequately describe the organic phosphorus (P) release in the studied soil. This is because these models failed to represent the real release pattern (Louhar et al., 2019).

The type of manure affects the P release rate of the Parabolic diffusion model. Our results indicated that the PM-treated soil had the highest release rate constant (kp) (4.92 (mg kg⁻¹)^{-0.5}) compared to FYM- and SM-treated soils (2.06 and 2.70 (mg kg⁻¹)^{-0.5}), respectively (Table 6). Chen et al. (2021) found that pig manure (PM) and organic compost (OM) tend to have higher initial P release rates than dairy dung (DM) and chicken manure (CM), particularly in flood-prone areas. Results in Table (6) indicate that all P release rate constants for all kinetic models increased in the inoculated soils with PSB compared to the un-inoculated ones in all the studied treatments. Our results in Table (6) indicated that the P release rate constant (*kp*) of Parabolic diffusion equations increased from 4.92, 2.06, and 2.70 (mg kg⁻¹)^{-0.5} in PM-. FYM-, and SM-treated soils to 5.32, 2.67, and 2.98 (mg

kg⁻¹)^{-0.5} in PM+PSB-, FYM+PSB-, and SM+PSBtreated ones, respectively. These findings prove the positive impact of PSB on improving and fasting the P release from the treated soils.

Table 5 : The linear regression equations of the kinetic models for P release description.

Treatment	Zero order	First order	Second order
CK	Y=0.0673x+7.4303	Y=0.0083x+1.9732	Y= -0.0011x+0.1434
CK+PSB	Y=0.0757x+9.2187	Y=0.0081x+2.1713	Y = -0.0009x + 0.1201
PM	Y=0.5672x+14.103	Y=0.0229x+2.6426	Y = -0.0011x + 0.0732
PM+PSB	Y=0.5936x+19.717	Y=0.0201x+2.9349	Y = -0.0008x + 0.0567
FYM	Y=0.2253x+23.707	Y=0.0080x+3.1591	Y = -0.0003x + 0.0427
FYM+PSB	Y=0.2868x+27.01	Y=0.0090x+3.2835	Y = -0.0003x + 0.0380
SM	Y=0.3082x+20.270	Y=0.0115x+3.0111	Y = -0.0004x + 0.0493
SM+PSB	Y=0.3338x+23.348	Y=0.0111x+3.1455	Y = -0.0004x + 0.0434
Treatment	Power function	Parabolic diffusion	Simple Elovich
CK	Y=0.1027x+1.9607	Y=0.6841x+6.1960	Y=0.7932x+7.4022
CK+PSB	Y=0.1165x+2.1267	Y=0.8173x+7.6360	Y=1.0373x+8.8996
PM	Y=0.2066x+2.7579	Y=4.9157x+7.1388	Y=4.5531x+18.0720
PM+PSB	Y=0.2093x+2.9810	Y=5.3201x+11.715	Y=5.3222x+22.7680
FYM	Y=0.0777x+3.1892	Y=2.0613x+20.499	Y=2.0832x+24.7390
FYM+PSB	Y=0.0944x+3.3020	Y=2.6680x+22.747	Y=2.8254x+27.9810
SM	Y=0.1009x+3.0737	Y=2.6927x+16.398	Y=2.5453x+22.2850
SM+PSB	Y=0.1075x+3.1872	Y=2.9774x+18907	Y=2.9741x+25.1020

Table 6: The kinetic parameters and determination coefficients (R²) of the studied models.

Tuesday	Zero order Fin			torder		Second order			
Treatment	K ₀	R ²	K ₁	R ²	K	2	F	2	
CK	0.0673	0.4907	0.0083	0.4781	- 0.00	011	0.4	0.4527	
CK+PSB	0.0757	0.3731	0.0081	0.3622	- 0.00)09	0.3	380	
PM	0.5672	0.9049	0.0229	0.8135	- 0.00	011	0.6	978	
PM+PSB	0.5936	0.8498	0.0201	0.7108	- 0.00	008	0.5	446	
FYM	0.2253	0.7392	0.0080	0.7266	- 0.00	003	0.7	013	
FYM+PSB	0.2868	0.7371	0.0090	0.6873	- 0.00	003	0.6	239	
SM	0.3082	0.8779	0.0115	0.8421	- 0.0004		0.7899		
SM+PSB	0.3338	0.8515	0.0111	0.7855	- 0.0004		0.7014		
Tuestment	Pov	wer funct	tion	Parabolic	diffusion	Sin	nple Elovich		
Treatment	K_f	b	R ²	kp	R ²	β	α	R ²	
CK	7.10	0.1027	0.8810	0.6841	0.7228	0.7932	7.4022	0.8250	
CK+PSB	8.39	0.1165	0.9034	0.8173	0.6204	1.0373	8.8996	0.8486	
PM	15.77	0.2066	0.8022	4.9157	0.9700	4.5531	18.072	0.7066	
PM+PSB	19.71	0.2093	0.9333	5.3201	0.9744	5.3222	22.768	0.8280	
FYM	24.27	0.0777	0.8233	2.0613	0.8835	2.0832	24.740	0.7662	
FYM+PSB	27.17	0.0944	0.9206	2.6680	0.9107	2.8254	27.981	0.8671	
SM	21.62	0.1009	0.7907	2.6927	0.9564	2.5453	22.285	0.7256	
SM+PSB	24.22	0.1075	0.8899	2.9774	0.9668	2.9741	25.102	0.8191	

Adnan et al. (2017) found that when compared to uninoculated controls, PSB inoculation dramatically increases P release, the effect is most noticeable during the first weeks of incubation. For instance, the greatest increases in available P were noted in the first 7–14 days after PSB treatment, particularly when combined with soluble P sources such single super phosphate (SSP), in a study that measured Olsen-extractable P over 56 days (Adnan et al., 2017). Compared to mineral sources alone, organic P sources and PSB frequently maintain greater P availability for longer periods of time (Liu et al., 2023).

2.6. In the *In vivo* study:

This study was conducted to quantify the population of phosphate-solubilizing bacteria after a 60-day incubation period in sandy soil amended with different organic matter sources, including poultry manure, farmyard residues, and sheep manure, while maintaining soil moisture at field capacity. The objective was to evaluate the potential relationship between the abundance of these bacteria and the concentration of available phosphorus in the soil. As shown in Table (7), the lowest population of phosphate-solubilizing bacteria was recorded in the soil without organic matter amendment. In contrast, a significant increase in bacterial counts was observed in treatments where organic manures were applied. The highest populations were found in soils amended with poultry manure (PM) and sheep manure (SM), with both showing comparable levels. This suggests that the increase in available phosphorus in the poultry manure (PM) treatment may be attributed not only to the enhanced activity of phosphate-solubilizing bacteria but also to the composition of the organic material itself. These findings are consistent with the results reported by Kucey (1983), who found that phosphate-solubilizing bacteria and fungi were more active in soils with organic amendments compared to unamended soils.

Treatments	PSB count CFU/g $(X10^4)$
CK	0.06
CK+PSB	0.18
PM	4.66
PM+PSB	7.81
FYM	3.93
FYM+PSB	5.62
SM	4.64
SM+PSB	7.36

Table 7. PS bacterial counts of all treatments after 60 days of inoculation.

CONCLUSION

Because of their high P fixation by calcium compounds and generally poor nutrient retention, sandy calcareous soils are characterized by low phosphorus (P) availability. For sustainable agriculture, increasing P availability in these soils is a major challenge. Relying on organic fertilizers such as poultry, farmyard, and sheep manures can significantly improve phosphorus availability in sandy calcareous soils by reducing soil pH, increasing labile P, enhancing microbial activity, and reducing P fixation. The integration use of phosphorus solubilizing bacteria with these organic manures enhances the P mineralization and release rate from these organic amendments. Adding phosphorus-dissolving bacteria with organic fertilizers does maximize the benefits of these fertilizers by increasing phosphorus availability in the sandy calcareous soil, reducing reliance on synthetic fertilizers and lowering input costs and environmental pollution. This approach supports sustainable agriculture and efficient nutrient management. More field trials are required to assess the effect of co-application of organic manures and phosphorus-solubilizing bacteria on phosphorus availability under field climatic conditions in sandy and calcareous soils.

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